Exciting progress of cancer immunotherapy focusing on immune checkpoints

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Abstract. Immune checkpoints blockade (ICB) has made revolutionary progress in cancer therapy recently. The development of blocking agents to checkpoints on coinhibitory pathway, which prevents inflammation-induced tissue damage but also induces the cancer immune evasion, and retrieves the productive immune responses against tumors. The striking clinical trial results of ICB, by targeting the cytotoxic T lymphocyte-associated protein 4 (CTLA-4), the programmed cell death 1 (PD-1) or PD-1 ligand 1 (PD-L1), has promoted the approval of multiple antibodies for several cancer types by the US Food and Drug Administration (FDA). In addition, the combination of multiple types of blockade even increased the efficacy of tumor regression. Following the previous success, other immune checkpoints have also been verified, such as lymphocyte-activated gene-3 (LAG-3) and Signal-regulatory Protein alpha (SIRP α). However, not all patients can get benefits from ICB and the mechanisms of these coinhibitory pathways are not quite clear. Therefore, understanding the mechanisms of ICB is a formidable challenge that could have far reaching guidelines for therapeutic strategies in cancer. This article summarizes the literature to date regarding ICB that may help more patients get benefit from immunotherapy.

1 INTRODUCTION

Immune checkpoints like CTLA-4, PD-1, LAG-3, and SIRP- α are discovered to be used by tumor cells as tools to suppress the immune system. Monoclonal antibodies were developed to block the signaling between those molecules and their ligands. Most of those Abs showed significant effect on the regression of the tumor cells on clinical trials and some of them were officially proved and created as commercials. In this article, we are going to introduce some of the immune checkpoints and their mechanisms, and present their functions as therapeutic targets.

2 CTLA-4

CTLA-4 (CD152) was discovered as a negative regulator for T-lymphocyte in 1985 and later identified in 1987. CTLA-4 is a homolog of T-lymphocytes co-stimulatory receptor CD28, and they bind to the same ligands B7-1(CD80) and B7-2(CD86), but CTLA-4 has a higher affinity to those ligands.[1]

CTLA-4 inhibits T cell response in an intrinsic and an extrinsic manner. (1) In a T cell, CTLA-4 can intrinsically outcompete with CD28 for B7 ligand binding or it recruits phosphatases to the cytoplasmic area of CTLA-4, which results in a reduced TCR and CD28 signaling.[2] (2) CTLA-4 on T regulatory cells can extrinsically inhibit the activation of other T cells by reducing the expression of B7-1 and B7-2 on Antigen Presenting Cells. T regulatory cells can release cytokines

like IL-10 causing the downregulation of B7[3] or remove them from APCs through trans-endocytosis, which results in a reduced interaction between CD28 and B7.[4]

Numerous studies have shown that anti-CTLA-4 blocking antibodies can facilitate tumor regression, long term immunity, and antitumor response. The anti-CTLA-4 blocking antibodies target both effector T cells and regulatory T cells; CD8⁺ T cells are always required for therapeutic benefit, but CD4⁺ T cells are only needed in some mouse models.[4] Recent works have shown two effects of anti-CTLA-4 blocking antibodies. They block the interaction of CTLA-4 pathways and decrease the number of regulatory T cells in the tumor microenvironment.[4,5]

The ratio of CD8⁺ TILs/Tregs has a positive correlation with overall survival of patients.[6] In a ovarian cancer microenvironment, CTLA-4 blocking antibody promoted the TILs growth in majority of patients (12/14) compared to 64% of patients when tumor fragments were cultured in IL-2 standard condition.[7] The two anti-CTLA-4 monoclonal antibodies ipilimumab and tremelimumab show great effects in immunotherapy and establish more ways to promote the depletion of tumors. Patients who received the combination of gp100 peptide vaccine and ipilimumab shows a higher survival advantage than only receiving the treating of gp100 peptide.[4,8] A second phase 3 trial, the combination of dacarbazine and ipilimumab compared to dacarbazine alone shows an overall greater survival rate at a 3 years to 1 year on

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patients with untreated metastatic melanoma.[9] Based on those studies, ipilimumab was approved for metastatic melanoma in the United States and Europe in 2011.[4] However, when comparing to chemotherapy, tremelimumab failed to reach a higher survival advantage for metastatic melanoma.[10]

Although the anti-CTLA-4 monoclonal antibodies indeed show a great effect on tumor immunity, patients often develop immune-related adverse events (irAEs) in tissues like skin, intestine, liver, and lung, which often have high microbial exposure.[11-13] Although irAEs can be fatal, corticosteroids and anti-inflammatory therapies can promote successful management of those toxicities in immune check point blockade.[4]

3 PD-1 AND PD-L1

Programmed death-1 (PD-1, CD279) was first discovered by Tasuku Honjo and his colleagues. Similar to CTLA-4, PD-1 serves as a negative regulator for immune response, by mediating T cell exhaustion, T cell activation, T cell tolerance, and T cell resolution of inflammation.[4,14] To achieve this, PD-1 binds to its ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273). When T cells receive repetitive stimulation from antigens in the setting of cancer and chronic infection, the level of PD-1 increases and T cells go through epigenetic modification, which leads to a state called exhaustion. T cells in exhaustion state tend to express other inhibitory receptors, making them susceptible to inhibition by multiple checkpoint pathways.[4,15] PD-1 can be expressed on other cells as well, on NK cells and B cells, limiting their effector function[16-18], on macrophages, suppressing their function during diseases like sepsis, and on T regulatory cells and T follicular regulatory cells, modulating their induction and function.[4,19]

The binding of PD-1 and PD-L1 or PD-L2 will cause the tyrosine phosphorylation of the PD-1 cytoplasmic area and recruitment of tyrosine phosphatase SHP-2, which leads to a reduced downstream of T cell receptor stimulation, decreased phosphorylation of TCR signaling molecules, and lower production of cytokines.[20] Signaling of PD-1 can also trigger expression of proteins that inhibit T cell proliferation and production of cytokines and increase the expression of proapoptotic gene like Bcl2l11 (Bim), thus decreasing the overall survival of T cells.[21] Lastly, T regulatory cells from naïve or Th cells can be induced by PD-1 pathway, which can suppress the T cell effector functions.[4,19] Overall, PD-1 pathway can downregulate the activity of T cells in many ways to inhibit the effector function of T cells.

Several PD-1 monoclonal antibodies are approved to show great effect on immunotherapy, leading to FDA approval. In 2014, nivolumab and pembrolizumab are approved for advanced refractory melanoma and in 2015, nivolumab is approved for advanced refractory squamous NSCLC and renal cell carcinoma.[4] Pembrolizumab is an anti-PD-1 antibody that is being used to evaluate patients with renal cell carcinoma (RCC) in a phase II trial KEYNOTE-427 (NCT02853344).[22]

Patients who received treatment with first-line pembrolizumab achieved an ORR of 38% and a disease control rate of 69% with a median duration of 12 months.[22,23] Atezolizumab, an anti-PD-L1 antibody, was used as a first-line single-agent for metastatic RCC in a phase I study (NCT01375842) and evaluated in a phase II IMmotion 150 trial (NCT01984242), including atezolizumab plus bevacizumab, atezolizumab alone or sunitinib.[22-24] Atezolizumab achieved an ORR of 25% compared with sunitinib of 29%.[22] A randomized comparison of PD-1 and CTLA-4 blockade in melanoma shows the better effect of pembrolizumab over ipilimumab, with a higher response rate, lower rate of irAEs (19.9% versus 13.3% and 10.1%).[25] More therapeutic trials indicate the benefits and tumor immunity of PD-1/PD-L1 blockade.

4 LAG-3

The coinhibitory receptor lymphocyte activation gene-3 (LAG-3, CD233) was discovered by Frédéric Triebel in 1990.[26] LAG-3 is expressed on CD4+ and CD8+ T cells, NK cells, NKT cells, and dendritic cells.[27] Its expression can be upregulated by interleukin (IL)-2 and IL-12 on activated T cells, where it mainly functions as a receptor that delivers inhibitory signals. LAG-3 can be also found on regulatory T-cells and helps to increase the suppressive ability of them.[28] LAG-3 downregulates the proliferation and function of T-cells and is associated with the exhaustion of T-cells. It is worthy to note that PD-1 and LAG-3 are often co-expressed in tumor microenvironment.[29]

LAG-3 is the homolog of CD4 that binds to MHC class II molecules, but with a higher affinity than CD4.[30] Except the canonical ligand MHC-II, other ligands have been discovered such as galectin-3, LSECtin, α -synuclein, and liver secreted protein fibrinogen-like protein 1 (FGL1). Among these ligands, FGL1 was recently demonstrated as the major ligand of LAG-3 that mediates T cell suppression.[31] FGL1 is normally released into the blood plasma by the liver in low levels but by cancer in high levels. Though the physiological functions of FGL1 in hepatocyte regeneration and metabolism are not well understood, blockade the interaction of FGL1-LAG-3 potentiates antitumor immunity in a receptor-ligand inter-dependent manner.[31] The function of LAG-3 depends on the signaling through its cytoplasmic domain KIEELE motif, but the downstream signaling mechanism is still not clear.[28,32]

Based on a study of murine LAG-3 Ig, Immutep (IMP321) was created and used to test in several clinical trials. In a study combined Immutep with taxane-based chemotherapy for women with breast cancer showed a result of increasing number and activation of APC and augmented number of NK cells and long-lived cytotoxic-memory CD8 T cell.[33] Additionally, the ORR was twice higher than the historical control group. There are at least ten clinical trials in development using LAG-3 monoclonal antibodies with combination with other agents like anti-PD-1 or anti-PD-L1.[28] The first

antagonistic mAb to LAG-3 was relatlimab. Combination of relatlimab with nivolumab receive an ORR of 11.5% in patients with advanced melanoma in a phase I-II study.[28,34] Patients with expression of LAG-3 by TILs shows a higher response rate than patients without expression of LAG-3 on TILs.[28,34] Research have made several bispecific antibodies to target both LAG-3 and PD-1/PD-L1. FS118, a bispecific monoclonal antibody, is composed with anti-PD-L1 with its Fc region structured by anti-LAG-3. Studies have shown FS118 helps to enhance the activation of CD8+ T cells compared to anti-PD-L1 alone.[28,35]

5 SIRPA AND CD47

Signal-regulatory Protein alpha (SIRP α) is an inhibitory receptor that expresses on macrophages, dendritic cells, and neutrophils.[36] SIRP α binds to its ligand CD47, which is broadly expressed on many normal cells, but significantly expressed on cancer cell.[36] The pathway of SIRP α -CD47 is associated with a process known as phagocytosis. Phagocytosis is a process that cells like macrophages engulf many objects like cell debris, pathogen, red blood cells and most importantly tumor cells. The engulfment of those objects results in phagolysosome in which the object is digested and destroyed. It can also produce antigenic peptides that are presented on MHC class molecules, and then cause an initial adaptive immune response. The binding of SIRP α -CD47 is the inhibitory signal to the macrophage.

Now, we understand the role of SIRP α -CD47 pathway in phagocytosis, but how does it operate under molecular level? The cytoplasmic domain of SIRP α has four tyrosine residues, which forms two ITIMs (immunoreceptor tyrosine-based inhibition motifs).[37] When they become phosphorylated, they recruit and activate inhibitory molecules, in particular tyrosine phosphatase SHP-1 and SHP-2, which can dephosphorylate many substrates and decrease the downstream signaling pathways.[36,37] Although this inhibitory cascade finally restrain the function of nonmuscle myosin IIA[38], the molecular intermediates via this pathway remain to be clarified. Overall, lack of binding of SIRP α -CD47 allows the activation of stimulatory receptors to trigger phagocytosis.

Several Abs and recombinant proteins, targeting CD47 or SIRP α , have been developed and are going through early clinical trials.[39] An anti-CD47 antibody, Hu5F9-G4, was shown to be efficiency, used either as single agent in patients with relapsed/refractory cancers in a phase I clinical trial[40], or in combination with the anti-CD20 monoclonal antibody rituximab for the treatment of relapsed or refractory non-Hodgkin lymphoma.[41] In addition, a pleiotropic anticancer agent in phase III clinical trials, RRx-001, has recently been recognized as a minimally toxic dual checkpoint inhibitor of CD47 and SIRP α that primes macrophages both in vitro and in vivo to attack cancer cells (Table 1).[42]

6 DISCUSSION

In recent years, many combinations of ICB were used for different types of cancers. One of the approaches for the future immunotherapy is to design better combinations of agents. However, ICB decreases the capability of the immune system to prevent autoimmunity and inflammation. New approaches should be to find receptors that are expressed specifically in the tumor sites, so the blockade can only happen in the tumor site.[11] Other researches that work on restricting tumor cells are developing strategies, including: (i) Vaccines targeting neoantigens[43-45]; (ii) better delivery of blocking agents to the tumor microenvironment; (iii) Chimeric antigen receptor T-cell therapy (CAR-T)[46]; (iv) bispecific antibodies[47-49]; and (v) Molecular shields restricting local activity of checkpoint inhibitors.[50] So, our scope of the future of immunotherapy is to suppress the activity of tumors while preventing autoimmunity.

Table 1. Summary of Immune Checkpoints Blockade

Target	Ligand	Agent	Reference
CTLA-4	CD80 CD86	ipilimumab tremelimumab	[7][9]
PD-1	PD-L1 PD-L2	Nivolumab Pembrolizumab Atezolizumab	[4][22-24]
LAG-3	MHC-II FGL1 galectin-3 LSECtin	Immutep (IMP321) relatlimab	[33]
SIRP α	CD47	Hu5F9-G4 RRx-001	[41] [42]

References

1. A. Collins, D. Brodie, R. Gilbert, et al. The interaction properties of costimulatory molecules revisited. *Immunity*, **17**(2), 201-10 (2002)
2. H. Bour-Jordan, H. Esensten, M. Martinez-Llordella, et al. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/ B7 family. *Immunol Rev*, **241**(1), 180-205 (2011)
3. K. Wing, Y. Onishi, P. Prieto-Martin, et al. CTLA-4 control over Foxp3+ regulatory T cell function. *Science*, **322**(5899), 271-5 (2008)
4. H. Baumeister, J. Freeman, G. Dranoff, et al. Coinhibitory Pathways in Immunotherapy for Cancer. *Annu Rev Immunol*, **34**, 539-73 (2016)
5. J. Selby, J. Engelhardt, M. Quigley, et al. Anti-CTLA-4 antibodies of IgG2a isotype enhance antitumor activity through reduction of intratumoral regulatory T cells. *Cancer Immunol Res*, **1**(1), 32-42 (2013)

6. E. Sato, H. Olson, J. Ahn, et al. Intraepithelial CD8+ tumor-infiltrating lymphocytes and a high CD8+/regulatory T cell ratio are associated with favorable prognosis in ovarian cancer. *Proc Natl Acad Sci U S A*, **102(51)**, 18538-43 (2005)
7. C. Friese, K. Harbst, H. Borch, et al. CTLA-4 blockade boosts the expansion of tumor-reactive CD8(+) tumor-infiltrating lymphocytes in ovarian cancer. *Sci Rep*, **10(1)**, 3914 (2020)
8. S. Hodi, J. O'day, F. McDermott, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*, **363(8)**, 711-23 (2010)
9. C. Robert, L. Thomas, I. Bondarenko, et al. Ipilimumab plus Dacarbazine for Previously Untreated Metastatic Melanoma. *New England Journal of Medicine*, **364(26)**, 2517-2526 (2011)
10. A. Ribas, R. Kefford, A. Marshall, et al. Phase III randomized clinical trial comparing tremelimumab with standard-of-care chemotherapy in patients with advanced melanoma. *J Clin Oncol*, **31(5)**, 616-22 (2013)
11. A. Schnell, L. Bod, A. Madi, et al. The yin and yang of co-inhibitory receptors: toward anti-tumor immunity without autoimmunity. *Cell Res* (2020).
12. H. June, T. Warshauer, A. Bluestone. Corrigendum: Is autoimmunity the Achilles' heel of cancer immunotherapy?. *Nat Med*, **23(8)**, 1004 (2017)
13. M. Michot, C. Bigenwald, S. Champiat, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*, **54**, 139-148 (2016)
14. T. Okazaki, S. Chikuma, Y. Iwai, et al. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. *Nat Immunol*, **14(12)**, 1212-8 (2013)
15. D. Blackburn, H. Shin, N. Haining, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol*, **10(1)**, 29-37 (2009)
16. M. Terme, E. Ullrich, L. Aymeric, et al. IL-18 induces PD-1-dependent immunosuppression in cancer. *Cancer Res*, **71(16)**, 5393-9 (2011)
17. R. Bellucci, A. Martin, D. Bommarito, et al. Interferon-gamma-induced activation of JAK1 and JAK2 suppresses tumor cell susceptibility to NK cells through upregulation of PD-L1 expression. *Oncoimmunology*, **4(6)**, e1008824 (2015)
18. V. Velu, K. Titanji, B. Zhu, et al. Enhancing SIV-specific immunity in vivo by PD-1 blockade. *Nature*, **458(7235)**, 206-10 (2009)
19. M. Francisco, H. Salinas, E. Brown, et al. PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. *J Exp Med*, **206(13)**, 3015-29 (2009)
20. E. Keir, J. Butte, J. Freeman, et al. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*, **26**, 677-704 (2008)
21. M. Gibbons, X. Liu, V. Pulko, et al. B7-H1 limits the entry of effector CD8(+) T cells to the memory pool by upregulating Bim. *Oncoimmunology*, **1(7)**, 1061-1073 (2012)
22. W. Xu, B. Atkins, F. McDermott. Checkpoint inhibitor immunotherapy in kidney cancer. *Nat Rev Urol*, **17(3)**, 137-150 (2020)
23. F. McDermott, J-L. Lee, C. Szczylik, et al. Pembrolizumab monotherapy as first-line therapy in advanced clear cell renal cell carcinoma (accRCC): Results from cohort A of KEYNOTE-427. *Journal of Clinical Oncology*, **36(15_suppl)**, 4500-4500 (2018)
24. F. McDermott, A. Sosman, M. Sznol, et al. Atezolizumab, an Anti-Programmed Death-Ligand 1 Antibody, in Metastatic Renal Cell Carcinoma: Long-Term Safety, Clinical Activity, and Immune Correlates From a Phase Ia Study. *J Clin Oncol*, **34(8)**, 833-42 (2016)
25. C. Robert, J. Schachter, V. Long, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*, **372(26)**, 2521-32. (2015)
26. F. Triebel, S. Jitsukawa, E. Baixeras, et al. LAG-3, a novel lymphocyte activation gene closely related to CD4. *J Exp Med*, **171(5)**, 1393-405 (1990)
27. B. Huard, P. Gaulard, F. Faure, et al. Cellular expression and tissue distribution of the human LAG-3-encoded protein, an MHC class II ligand. *Immunogenetics*, **39(3)**, 213-7 (1994)
28. P. Andrews, H. Yano, A. Vignali. Inhibitory receptors and ligands beyond PD-1, PD-L1 and CTLA-4: breakthroughs or backups. *Nat Immunol*, **20(11)**, 1425-1434 (2019)
29. P. Andrews, E. Marciscano, G. Drake, et al. LAG3 (CD223) as a cancer immunotherapy target. *Immunol Rev*, **276(1)**, 80-96 (2017)
30. E. Baixeras, B. Huard, C. Miossec, et al. Characterization of the lymphocyte activation gene 3-encoded protein. A new ligand for human leukocyte antigen class II antigens. *J Exp Med*, **176(2)**, 327-37 (1992)
31. J. Wang, F. Sanmamed, I. Datar, et al. Fibrinogen-like Protein 1 Is a Major Immune Inhibitory Ligand of LAG-3. *Cell*, **176(1-2)**, 334-347.e12 (2019)
32. B. Huard, R. Mastrangeli, P. Prigent, et al. Characterization of the major histocompatibility complex class II binding site on LAG-3 protein. *Proc Natl Acad Sci U S A*, **94(11)**, 5744-9 (1997)
33. C. Brignone, M. Gutierrez, F. Mefti, et al. First-line chemoimmunotherapy in metastatic breast carcinoma: combination of paclitaxel and IMP321 (LAG-3Ig) enhances immune responses and antitumor activity. *J Transl Med*, **8**, 71 (2010)
34. P. Cottu, V. D'hondt, S. Dureau, et al. LBA9Letrozole and palbociclib versus 3rd generation chemotherapy as neoadjuvant treatment of minal breast cancer. Results of the

- UNICANCER-eoPAL study. *Annals of Oncology*, **28(suppl_5)** (2017)
35. M. Kraman, N. Fosh, K. Kmieciak, et al. Abstract 2719: Dual blockade of PD-L1 and LAG-3 with FS118, a unique bispecific antibody, induces CD8+ T-cell activation and modulates the tumor microenvironment to promote antitumor immune responses. *Cancer Research*, **78(13 Supplement)**, 2719-2719 (2018)
36. A. Veillette, J. Chen. SIRPalpha-CD47 Immune Checkpoint Blockade in Anticancer Therapy. *Trends Immunol*, **39(3)**, 173-184 (2018)
37. N. Barclay, K. Van Den Berg. The interaction between signal regulatory protein alpha (SIRPalpha) and CD47: structure, function, and therapeutic target. *Annu Rev Immunol*, **32**, 25-50 (2014)
38. K. Tsai, E. Discher. Inhibition of "self" engulfment through deactivation of myosin-II at the phagocytic synapse between human cells. *J Cell Biol*, **180(5)**, 989-1003 (2008)
39. A. Veillette, Z. Tang. Signaling Regulatory Protein (SIRP)alpha-CD47 Blockade Joins the Ranks of Immune Checkpoint Inhibition. *J Clin Oncol*, **37(12)**, 1012-1014 (2019)
40. I. Sikic, N. Lakhani, A. Patnaik, et al. First-in-Human, First-in-Class Phase I Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients With Advanced Cancers. *J Clin Oncol*, **37(12)**, 946-953 (2019)
41. R. Advani, I. Flinn, L. Popplewell, et al. CD47 Blockade by Hu5F9-G4 and Rituximab in Non-Hodgkin's Lymphoma. *N Engl J Med*, **379(18)**, 1711-1721 (2018)
42. P. Cabrales. RRx-001 Acts as a Dual Small Molecule Checkpoint Inhibitor by Downregulating CD47 on Cancer Cells and SIRP- α on Monocytes/Macrophages. *Transl Oncol*, **12(4)**, 626-632 (2019)
43. F. Chen, Z. Zou, J. Du, et al. Neoantigen identification strategies enable personalized immunotherapy in refractory solid tumors. *J Clin Invest*, **129(5)**, 2056-2070 (2019)
44. C. Nonomura, M. Otsuka, R. Kondou, et al. Identification of a neoantigen epitope in a melanoma patient with good response to anti-PD-1 antibody therapy. *Immunol Lett*, **208**, 52-59 (2019)
45. N. Mcgranahan, C. Swanton. Neoantigen quality, not quantity. *Sci Transl Med*, 2019, **11(506)**.
46. H. June, S. O'connor, U. Kawalekar, et al. CAR T cell immunotherapy for human cancer. *Science*, **359(6382)**, 1361-1365 (2018)
47. M. Messaoudene, P. Mourikis, J. Michels, et al. T-cell bispecific antibodies in node-positive breast cancer: novel therapeutic avenue for MHC class I loss variants. *Ann Oncol*, **30(6)**, 934-944 (2019)
48. I. Koopmans, D. Hendriks, F. Samplonius, et al. A novel bispecific antibody for EGFR-directed blockade of the PD-1/PD-L1 immune checkpoint. *Oncoimmunology*, **7(8)**, e1466016 (2018)
49. H. Chang, Y. Wang, R. Li, et al. Combination Therapy with Bispecific Antibodies and PD-1 Blockade Enhances the Antitumor Potency of T Cells. *Cancer Res*, **77(19)**, 5384-5394 (2017)
50. S. Pai, M. Simons, X. Lu, et al. Tumor-conditional anti-CTLA4 uncouples antitumor efficacy from immunotherapy-related toxicity. *J Clin Invest*, **129(1)**, 349-363 (2019)